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Risks for Human Health of Using Wastewater for Turf Grass Irrigation

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1. Introduction

In recent years, continuous population growth in most Mediterranean countries such as Spain has caused an increase in consumption of existing water resources. This population increase has not only increased freshwater demand but has also increased the volume of wastewater generated (Quian and Mecham, 2005). Thus, there is an urgent need to conserve and protect freshwater and to utilize the wastewater generated (Gan et al., 2006). Using treated wastewater for agricultural irrigation helps to alleviate demand of scarce potable water and groundwater resources (Angin et al., 2005). Water scarcity and water pollution pose a critical challenge in many developing countries and it is difficult for authorities to manage water supplies and wastewater (Chizuru et al, 2005). There is a significant absence of legislation in the EU in controlling wastewater reuse in agriculture. Currently there are no international standards except for the Worldwide Health Organization Guidelines, which are starting points for setting water quality standards, including microbiological standards (Campos, 2008). The World Health Organization (WHO), the US Environmental Protection Agency (USEPA) and the World Bank have reviewed the public health aspects of crop irrigation with domestic wastewater and have made recommendations for the microbiological quality of treated wastewaters used for this purpose (Shuval et al. 1989; WHO 1989; USEPA 1992). A Spanish law (R.D 1620/2007) establishes limits on the use of wastewater depending on the type of application in which the irrigation of green spaces (sports fields, parks) and gardens (private and public) have been considered. Wastewater can be a resource but may present a hazard at the same time (WHO, 2006). Proper wastewater reuse may offer solutions for meeting water resource needs. The fundamental precondition for water reuse is that applications will not cause unacceptable public risks (Chizuru et al, 2005). According to Dr. John Sheaffer, the president of Sheaffer International, Ltd., (McKenzie, 2005) "Wastewater can be viewed as a resource, fresh water containing plant nutrients (nitrogen, phosphorus, and potassium). In the groundwater, these nutrients are a pollutant, but on a growing crop or turf, they are a resource. When wastewater is reused, it is not available to pollute the groundwater supply."

A common type of recycled water is water that has been reclaimed from municipal wastewater (sewage). Different, specific parameters must be analyzed depending on the origin of the wastewater and the intended use. Simple parameters such as salinity, *E. coli*, turbidity, TSS, organic matter, DOC and other N- and P-related variables offer useful information depending on the final use of the reclaimed water (Salgot et al, 2006).

The quality of irrigation water is of particular importance in arid zones where extremes in temperature and low relative humidity result in high evaporation rates, with consequent deposition of salt that tends to accumulate in the soil profile (Pescod, 1992).

The use of wastewater irrigation in turf grass could be an alternative to drinking water irrigation since several studies confirm that treated wastewater can be used for turf grass irrigation with a minimal environmental impact (Wu et al., 1996; Barton et al., 2005; Menzel and Broomhall, 2005; Lockett, 2008; Castro et al, 2011).

Otherwise, wastewater treatment plants (WWTP) represent a common source of odor emissions. Odor generated by wastewater using a sprinkler system to irrigate a park or public garden may condition use because of the nuisance it causes to the population. There are many studies on this topic (Lawrence & Tan, 1990; Capelli et al, 2009; Cheng et al, 2009; Bo et al, 2011), but it is difficult to avoid the problem unless effective disinfection treatment is used. The selection of which method works best depends on the concentration of the odor causing compounds, the air flow rate, available land area for the system, capital budget and discharge limitations for wastewater from the system.

Coliform bacteria are organisms present in the environment and in the feces of all warm-blooded animals and humans. They will not likely cause illness, but their presence in drinking water indicates that disease-causing organisms (pathogens) could be in the water system. Most pathogens that can contaminate water supplies come from human or animal feces. There are three different groups of coliform bacteria, each with a different level of risk: total coliform, faecal coliform, and *E. coli*. The total coliform group is a large group of different kinds of bacteria. Faecal coliforms are types of coliforms that mostly exist in feces and *E. coli* is a subgroup of faecal coliforms. Most *E. coli* bacteria are harmless and are found in great quantities in the intestines of people and warm-blooded animals. Some strains, however, can cause illness. This is the case of Enterohaemorrhagic *E. coli* (EHEC), recently occurred in Germany. It can cause diarrhea, severe stomach cramps and fever (Davis, 2011). The presence of *E. coli* in a drinking water sample almost always indicates recent fecal contamination, meaning there is a greater risk that pathogens are present (DOH, 2007). Total coliform bacteria are commonly found in the environment (e.g., soil or vegetation) and are generally harmless. If only total coliform bacteria are detected in drinking water, the source is probably environmental and faecal contamination is not likely. However, if environmental contamination can enter the system, there may also be a way for pathogens to enter the system.

Another test parameter is helminth populations, which are multicellular organisms. The free-living larvae are not usually pathogenic and they have high resistance to adverse environmental conditions and disinfectants. They are well adapted to survive in water systems and in some cases they emerge alive from domestic taps (Campos, 2008). *Ascaris* (a nematode) is the most common helminth egg in wastewater and sludge. Eggs contained in wastewater are not always infective. To be infective they need to develop larva, for which a certain temperature and moisture are required (26° C and 1 month in laboratory conditions). These conditions are usually found in soil or crops where eggs, deposited through irrigation with wastewater or sludge, can develop larva in 10 days. Helminth ova (or Helminth eggs) can live in water, soil, and crops for several months/years (Feachem et al., 1983 cited by Jimenez, 2007). In previous studies, sewage sludge and wastewater from the Wastewater Treatment Plant (WWTP) of the city of Albacete was determined to be safe and adequate for agricultural uses (de las Heras et al, 2005; Mañas, 2006; Mañas et al, 2010). However, risks to human health derived from microbial content in this type of water resource have not been well evaluated. Therefore, the main goals of the present study were: to make a replicate of a garden or a

public park irrigated with treated wastewater to evaluate the applicability of treated wastewater for turf grass by assessing the physical and chemical effects of continued usage of treated water on the soil and plant over a two-year period and to assess the human health risk and, if possible, to define the availability of wastewater for this use.

2. Materials and methods

2.1 Design of experiment and localization

The field trial was carried out in two square study plots, of 225 m² each, on farmland near the WWTP (Figure 1) of Albacete (165,000 inhabitants) in SE Spain (39° 00' 57.82" N; 1° 50' 50.45" W). The source of city drinking water was surface water at the time of the study. Two types of water treatments were considered: drinking water (D), considered the control, and treated wastewater (W). The origin of wastewater was mixed: approximately 70% of domestic origin and 30% industrial from two industrial areas. Most of these industries are related to knife manufacturing, automobile replacement parts and a few food producers.



Fig. 1. Location of the experiment in the wastewater treatment plant of Albacete

The city wastewater treatment plant uses trickling filters with an open-air system. The trickling filters consist of a plastic medium over which wastewater flows downward and causes a film of microbial slime to cover the medium bed. Filtered wastewater is poured into

a channel that, for years, local farmers have mainly used to irrigate their corn and winter cereal cultivations using a flooding irrigation system.

The study was conducted May 2007-August 2008 (during 466 days after planting). Rainfall (mm), monthly ET_0 (mm), mean average temperature ($^{\circ}C$) and sunlight ($MJ\ m^{-2}$) during the study period are shown in Figures 2 and 3.

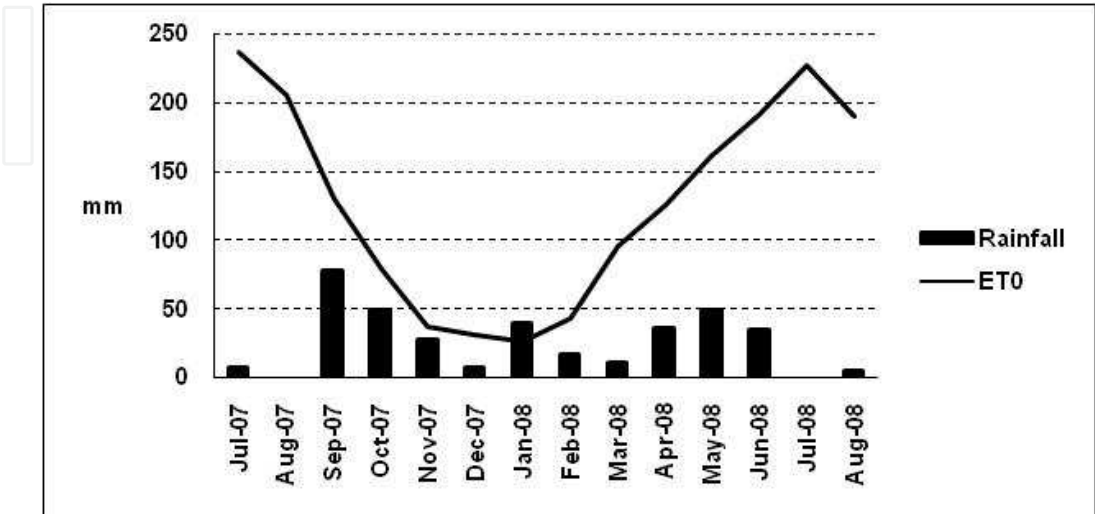


Fig. 2. Rainfall(mm) and ET_0 (mm) during the study period.

Throughout this period the mean minimum, mean maximum and mean average temperatures were $8.1^{\circ}C$, $23.0^{\circ}C$ and $15.5^{\circ}C$, respectively, total precipitation was 373.8 mm, average solar radiation was $20.3\ MJ\ m^{-2}$ and mean daily sunshine was 10.9 hours.

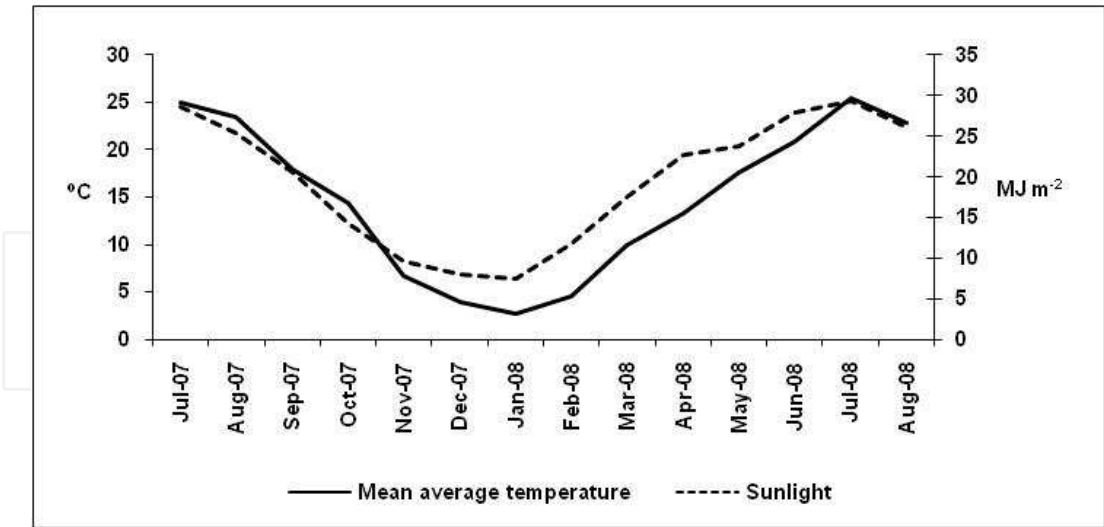


Fig. 3. Mean average temperature ($^{\circ}C$) and sunlight ($MJ\ m^{-2}$) during the study period.

At the beginning of the first year, a sprinkler irrigation system was installed in each plot corner ($15\ m \times 15\ m$) and the land was prepared with suitable farm machinery in order to apply 12 mm of mulch. One of the plots (control) was irrigated with drinking water and the other received treated wastewater (Figure 4). Each plot was divided into four sites (replicates).

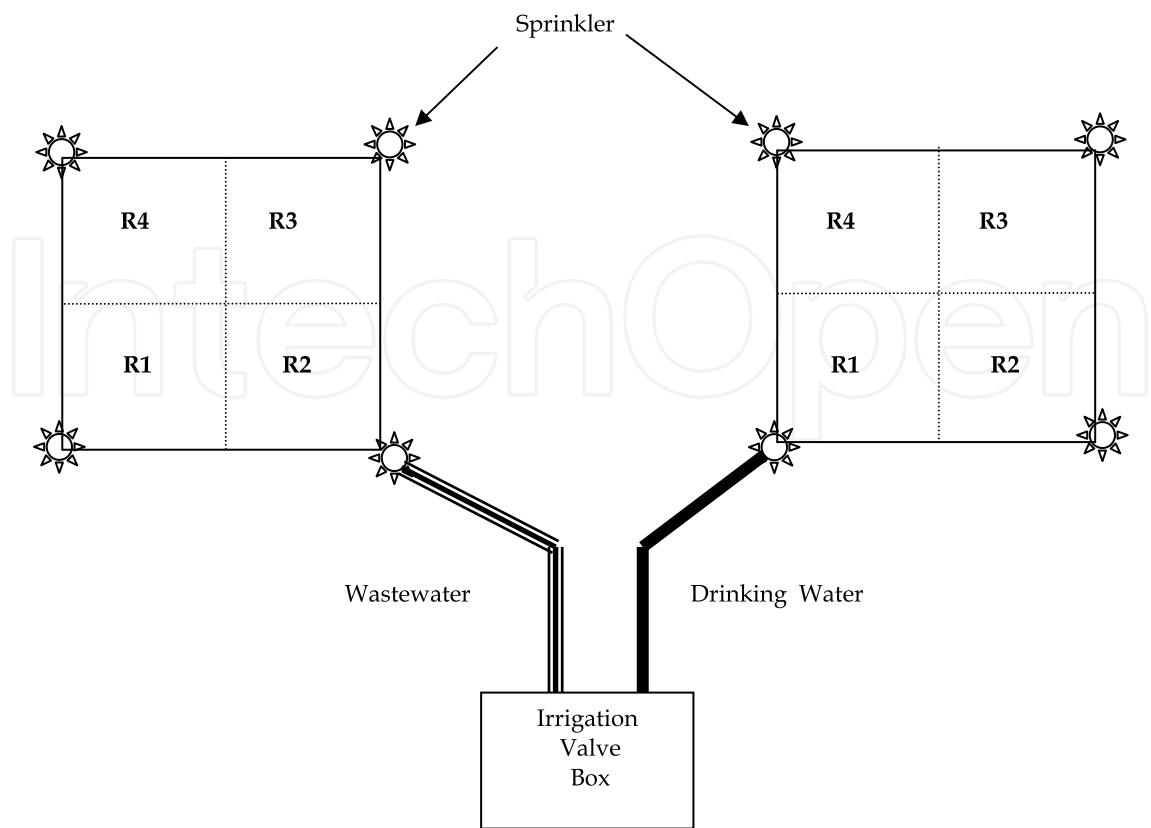


Fig. 4. Sketch of the experimental design. (R1, R2, R3 and R4: Replicates 1, 2, 3 and 4).

2.2 Sowing and Irrigation

In May, 2007, 35 g m⁻² of grass seed mixture was planted. The composition of the mixture was 65% of *Festuca Arundinacea* Kilimanjaro, 20% *Festuca Arundinacea* Arid 3,5% *Poa Pratense* Conni and 10% *Lolium perenne* Delaware. Weed control was performed using 2-methyl-4-chlorophenoxyacetic acid (MCPA). The plots were irrigated regularly to avoid drought stress. To calculate the water needs for this crop, we followed the methodology proposed by the FAO (Doorenbos & Pruitt, 1984), which calculates the Reference Evapotranspiration (ET_o) using the Penman-Monteith method and applies the crop coefficient (K_c). Since the ET_o and K_c values are known, Evapotranspiration and therefore water needs could be calculated. A total of 1688 mm of both types of water were distributed in irrigation. During the winter season (October 2007-May 2008), the crop was not irrigated because in this period the crop ceased growth and the risk of frost could damage both the crop and the irrigation system.

2.3 Sampling

2.3.1 Water

Treated wastewater and drinking water were tested ten times while the crop was in the field. The chemical composition of irrigation water (D and W) and some microbiological parameters (total coliforms, faecal coliforms, *Salmonella s.p* faecal streptococci and sulphite-reducing clostridia) were determined and recorded (Table 1). In drinking water, BOD₅, TSS and microbiological parameters were not tested. The composition of the two types of irrigation water does not vary significantly over the study period, so the mean values ± standard deviation for the chemical properties have been presented in both cases except for helminth eggs.

PARAMETER	UNITS	DRINKING WATER	TREATED WASTEWATER
		Average±sd	Average±sd
COD	mg l ⁻¹ O ₂	<10	125±86
BOD ₅	mg l ⁻¹ O ₂	Not detected	31.7±21.3
TSS	mg l ⁻¹	Not detected	23±18
pH		8.1±0.2 a	7.9±0.3 a
E.C.	μS cm ⁻¹	815±80 a	1759±237 b
Total water hardness	mg l ⁻¹	488±70	NT
Dissolved oxygen	mg l ⁻¹ O ₂	NT	2.5±1.7
Phosphorus	mg l ⁻¹	NT	4.2±1.4
N-Kjeldahl	mg l ⁻¹	NT	58.3±31.7
N-Ammoniacal	mg l ⁻¹	NT	31.6±10.7
Nitrite	mg l ⁻¹	<0.01	4.4±1.4
Nitrate	mg l ⁻¹	1.7±0.8 a	4.8±0.9 b
Sulphate	mg l ⁻¹	336±66 a	344±72 a
Carbonate	mg l ⁻¹	NT	46.3±15.5
Bicarbonate	mg l ⁻¹	NT	332±43
Chloride	mg l ⁻¹	28.6±4.5	242±7
IC	mg l ⁻¹	26.9±0.4 a	83.6±42.5 b
TC	mg l ⁻¹	29.5±0.9 a	104±50 b
TOC	mg l ⁻¹	2.5±0.5 a	20.2±7.5 b
SAR		0.2±0.1 a	2.5± 1.1b
Na	mg l ⁻¹	13.6±3.0 a	170±98 b
K	mg l ⁻¹	2.1±0.08 a	43.6±54.3 b
Ca	mg l ⁻¹	112±22 a	138±50 a
Mg	mg l ⁻¹	50.2±8.6 a	56.1±24.4 a
Zn	mg l ⁻¹	<0.24	0.5±0.4
Al	μg l ⁻¹	16.2±4.5 a	2786±1582 b
Cu	μg l ⁻¹	3.9±3.2 a	200±163 b
Fe	μg l ⁻¹	23.6±14.4 a	2120±1249 b
Pb	μg l ⁻¹	<7.5	31.1±22.4
Cd	μg l ⁻¹	<1	2.1±2.8
Cr	μg l ⁻¹	<5	30.1±20.4
Mn	μg l ⁻¹	8.2±6.8 a	62.3±66.8 b
Ni	μg l ⁻¹	<10	54.9±60.2
As	μg l ⁻¹	<10	<10
Se	μg l ⁻¹	<5	<5
B	μg l ⁻¹	0.03±0.02 a	0.3±0.1 b
Hg	μg l ⁻¹	<1	1.9±1.4
Total coliforms	cfu 100 ml ⁻¹	NT	1.7 104 ± 3.1 104
Faecal coliforms	cfu 100 ml ⁻¹	NT	4.1 103 ± 7.7 103
Salmonella sp	cfu 100 ml ⁻¹	NT	Not detected
Faecal streptococci	cfu 100 ml ⁻¹	NT	3.1 103 ± 3.0 103
Sulphite-reducing clostridia	cfu 100 ml ⁻¹	NT	2.4 103 ± 1.5 103
Helminths eggs	Eggs 10 l ⁻¹	NT	6

Table 1. Chemical composition of irrigation water. COD: Chemical Oxygen Demand; BOD₅: Biological Oxygen Demand, Five-Day; TSS: Total Suspended Solids; EC: Electrical Conductivity; IC: Inorganic Carbon; TC: Total Carbon; TOC: Total Organic Carbon;SAR: Sodium Absorption Ratio. NT: Not Tested. Different letters mean significant differences at p<0.05, according to Fisher’s LSD test. (n=10).

In addition, in order to describe chemical water data, we constructed Piper diagrams for D and W water (Figure 5). Microorganism presence in drinking water was not analyzed because it was chlorinated. In addition, treated wastewater was tested once in order to analyze for odor (APHA, 1998).

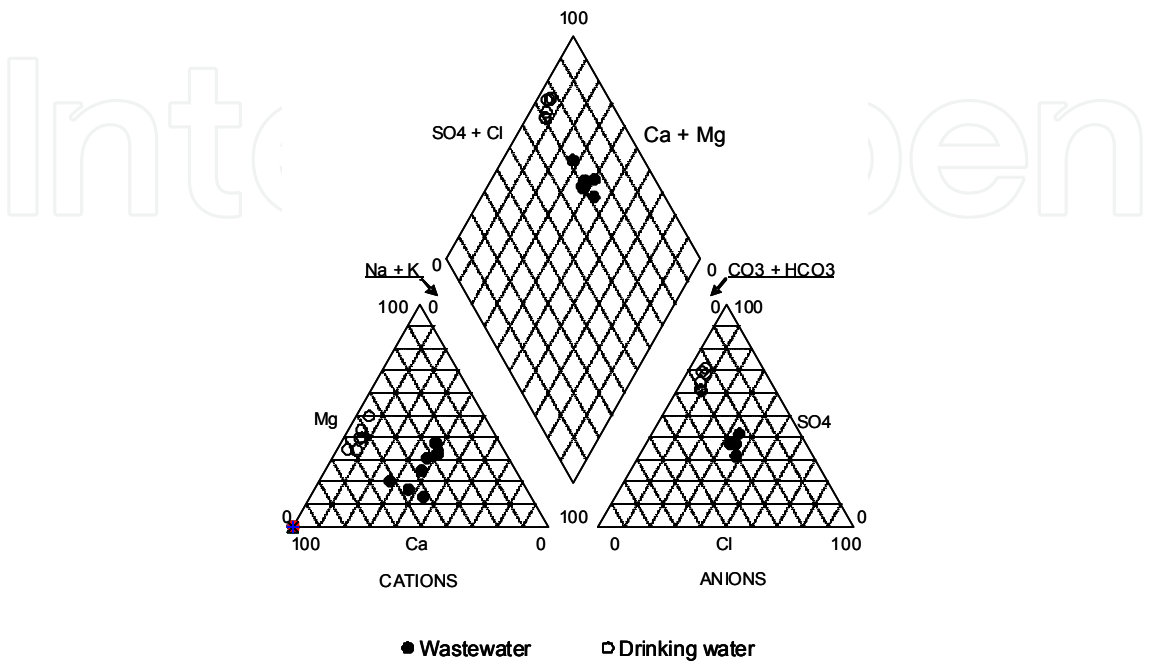


Fig. 5. Piper diagrams for irrigation water: wastewater and drinking water.

2.3.2 Soil

Before sowing a 25 cm deep soil sample from ten random points in the test plots was collected with a hand auger and three replicates were analyzed (n=3). At the end of the study (two months after suspending irrigation) new samples were collected at two different depths: 0-25 cm and 25-40 cm. In this case, soil samples were collected from ten points at random inside each of the four test plots and three repetitions of each were analyzed (S.A.F., 2005).

The original soil (Table 2) was basic, slightly saline (Cros, 1983), with a medium level of chloride, sulphate, organic matter and total nitrogen (Yañez, 1989). According to the C:N ratio, there was high nitrogen liberation (Quemener, 1985; Guigou et al. 1989). The amount of P, K, Ca and Mg was very high (Yañez, 1989). The total carbonate percentage was high but sodium content was low. The K:Mg and Ca:Mg ratios had adequate values (Yañez, 1989).

2.3.3 Grass

Height (cm) and phytomass growth (kg ha⁻¹) in wet weight were recorded six times during the crop season (summer-autumn 2007 and spring-summer 2008) and grass was mowed twice according table 3.

In winter 2007-2008 no plant samples were collected because crop growth was very slow and no differences had been observed. All measurements were collected randomly for one plant in each plot replicate (n=4).

PARAMETERS	UNITS	May 2007
		Original soil
		0-25 cm
Sand	%	27
Silt	%	29
Clay	%	45
pH		8.12
E.C	mmhos cm ⁻¹	0.69
Chloride	(mg gypsum) (100 g soil) ⁻¹	27
Sulphate	(mg gypsum) (100 g soil) ⁻¹	52
Organic matter	%	2.7
Total N	%	0.18
C:N Ratio		9
Nitric N	mg kg ⁻¹	47
Asimilable P	mg kg ⁻¹	66
Assimilable K	meq 100g ⁻¹	2.08
Assimilable Na	meq 100g ⁻¹	0.84
Assimilable Ca	meq 100g ⁻¹	24.51
Assimilable Mg	meq 100g ⁻¹	5.40
Assimilable B	mg kg ⁻¹	2.53
K:Mg Ratio		0.39
Ca:Mg Ratio		5

Table 2. Chemical characteristics of the original soil before sowing at 0-25 cm.

Date	Event
18/05/07	Sowing
15/07/07	Sampling
26/09/07	Sampling
24/10/07	Sampling
26/05/08	Mowing
02/06/08	Sampling
22/06/08	Sampling
26/06/08	Mowing
31/07/08	Sampling

Table 3. Dates of mowing and height and phytomass growth sampling events.

To get height and phytomass yields, a grass height meter developed by NMI (Nutrient Managament Institute) and distributed by Eijkelkamp was used to take measurements.

Following the NMI method, thirty measurements from each subplot were recorded to get an average value. Height was derived from the average of four replicate values. The NMI method determines phytomass (kg ha⁻¹) by indirect measurements according to the equation (1):

$$\text{Phytomass (kg ha}^{-1}\text{)} = 168.24 \times \text{Height (cm)} + 813.19$$

(1)

Therefore, the height value from each replicate (n=4) was used in this equation in order to determine phytomass. Finally, at the end of the last year foliar tissue samples from each treatment (n=4) were collected to determine chemical parameters such N, P, K, Ca, Mg, Na, Fe, Cu, Mn, Zn and Al.

Microbiological parameters (total coliforms, faecal coliforms, *Salmonella* sp. faecal streptococci and sulphite-reducing Clostridia in foliar tissue were tested eight times from July, 2007 to August, 2008 (Table 4) and a sample of each four-treatment replicate was collected in sterile plastic bags.

Date	Days after planting
18/05/07	0
15/07/07	58
26/09/07	131
24/10/07	159
07/04/08	323
26/05/08	372
22/06/08	399
31/07/08	438
28/08/08	466

Table 4. Dates of microbiological sampling events.

2.4 Analytical methods

2.4.1 Water

Water samples were analyzed with atomic emission (instrument error < 1%) to determine Na and K, atomic absorption spectroscopy (instrumental error <10%) to determine Fe, Al, Cu, Mn, Cd, Cr, Ni, Pb, As and Se and ionic chromatography (instrument error < 10%) to determinate chloride, sulphate and nitrate content.

Following the APHA (1998) method, COD in wastewater was determined by the open reflux method, BOD by 5-Day BOD test, pH by the electrometric method with a previous calibrated CRISON® GLP22 pH meter, TSS by membrane filter technique and EC by conductimetry.

Wastewater odor was tested according to the APHA (1998) method. To ensure precision we used a panel of 10 testers, starting with the most dilute sample first (1:200) to avoid affecting sensitivity with the concentrated sample (1:1). To assess microbiological content in wastewater, the most probable number count methodology was used (APHA, 1998). To determine total coliforms, faecal (F-) coliforms, faecal (F-) streptococci and sulphite-reducing (sr) Clostridia, dissolution from 25 g of fresh sample in tryptone water was prepared. The

growth media used were lactose broth for total coliforms and faecal (F-) coliforms, Rothe broth for streptococci and sulphite iron Wilson Blair agar for Clostridia. In every case the incubation period was 24 hours and the temperature was 37°C. To confirm total coliform (gas presence), lactose broth was used, incubating it during 24 hours at 37°C. To confirm faecal (F-) coliforms, E.C. broth was used as a growing medium and the incubation period was 24 hours at 44°C. Finally, to confirm streptococci growth, the medium was Bromocresol purple azide broth with the same incubation period and temperature as for total coliforms. To determine *Salmonella sp.*, the dissolution was prepared from 25 g of fresh sample in buffered peptone water. The growing medium was Rappaport broth and the incubation period was 24 hours at 42°C. To confirm *Salmonella sp.* presence HE Agar (Hekton Enteric) was used.

Helminth eggs were determining by counting number of eggs per 10 liters of water.

2.4.2 Soil

Soil samples were analyzed with the following techniques: pH with a previously calibrated CRISON® GLP22 pH meter and 1:2 w v-1 suspension of soil in water (Peech, 1965), organic matter using the Walkey Black method (Allison, 1965), electrical conductivity (Bower and Wilcox, 1965), N by the Kjeldahl procedure (Bremner, 1965) and extractable P (Olsen, 1965). Besides, soil samples (0.5 g dry weight) were prepared for analysis with acid digestion in 4 ml of HNO₃, 0.25 ml of H₂O₂ and 2 ml of HF and by applying the temperature program according to Milestones'® Cookbook of microwave application notes for MDR technology in order to determine Ca, Mg, and K (atomic emission in Spectr AA 50 Atomic Absorption Spectrometer with SIPS-10, Varian®); Fe, Zn, Al, Cu, Cd, Cr, Ni, Pb, As, and Mn (atomic absorption spectroscopy in Atomic Absorption Spectrometer Spectr. AA 220, Varian®).

2.4.3 Grass

Plant samples (0.5 g dry weight) were prepared for analysis with acid digestion in 6 ml of HNO₃, 1 ml of H₂O₂ and 0.5 ml of HF and we applied the temperature program according to Milestones'® Cookbook of microwave application notes for MDR technology. Next, we used atomic absorption spectroscopy analysis to determine K (d.l.: 0,01 ppm), Zn (d.l.: 0,1 ppm), Mg (d.l.: 0,05 ppm), Ca (d.l.: 0,1 ppm), Al (d.l.: 2 ppm), Cu (d.l.: 0,5 ppm), Fe (d.l.: 0,5 ppm) and Mn (d.l.: 0,2 ppm). N in plant samples was analyzed using the Kjeldahl Method and total P was determined by spectrophotometry after acid digestion in HNO₃ and H₂SO₄.

To determine microbiological parameters in foliar tissue, the same technique that in water samples was used.

2.5 Statistical procedures

The experimental design used 2 treatments with 4 replicates of turf grass. Data were subject to Anova treatments and the method used to discriminate among the means was Fisher's least significant difference (LSD) for $p < 0.05$. To ensure that data came from a normal distribution, standardized skewness and standardized kurtosis values were checked. Percentage values were transformed by arcsine. The dynamics of microorganisms in turf grass leaves were tested using a simple regression analysis. All statistical calculations were performed with Statgraphics plus 5.1.

3. Results and discussion

3.1 Water

During the study, 1688 mm of both types of water were distributed in irrigation. Important agricultural water quality parameters include a number of specific water properties that are relevant in relation to crop yield and quality, maintenance of soil productivity and environmental protection. These parameters mainly consist of certain physical and chemical characteristics of water (Pescod, 1992). Table 1 shows that electrical conductivity (E.C.), COD (Chemical Oxygen Demand), nitrite, nitrate, bicarbonate, chloride, inorganic carbon (IC), organic total carbon (TOC), total carbon (TC), sodium, potassium, aluminum, copper, iron, lead, cadmium, chrome, manganese, nickel and mercury have significantly higher values in wastewater than in drinking water. In addition, average B content in wastewater is 0.3 mg l⁻¹, which is excellent even for a crop sensitive to boron (Ayers and Westcot, 1987). The Piper diagram (Figure 5) shows than wastewater is a NaCa-SO₄Cl water type and drinking water corresponds to a Ca-SO₄ facie. On the other hand, Ayers and Westcott (1987) suggested some guidelines for interpreting water quality for irrigation based on Salinity, SAR, Specific Ion Toxicity (Na, Cl, B and trace elements) and miscellaneous effects, and they defined some degrees of restriction on usage. In Table 5, and according these authors, we can see that drinking water has no degree of restriction on use for sodium, chloride, boron and nitrogen and slight to moderate because of SAR and electrical conductivity. In contrast, treated wastewater has a slight to moderate degree of restriction on use for electrical conductivity, SAR, sodium, and chloride but no degree of restriction on use for boron or nitrogen.

	Drinking Water	Degree of Restriction on Use	Treated Wastewater	Degree of Restriction on Use
Electrical Conductivity (dS cm ⁻¹)	0.815	Slight to moderate	1759	Slight to moderate
SAR	0.2	Slight to moderate	2.5	Slight to moderate
Sodium (Na) meq l ⁻¹ Sprinkler irrigation	0.59	None	7.39	Slight to moderate
Chloride (Cl) meq l ⁻¹ Sprinkler irrigation	0.81	None	6.81	Slight to moderate
Boron (B) mg l ⁻¹	0.03	None	0.3	None
Nitrogen (NO ₃ – N) mg l ⁻¹	1.7	None	4.8	None

Table 5. Interpretations of water quality for irrigation according Ayers and Westcot (1987).

The presence of microorganisms in wastewater from highest to lowest was total coliforms (1.7 10⁴ cfu 100 ml⁻¹), faecal coliforms (4.1 10³ cfu 100 ml⁻¹), faecal streptococci (3.1 10³ cfu 100 ml⁻¹) and sulphite-reducing Clostridia (2.4 10³ cfu 100 mL⁻¹). *Salmonella sp.* was not detected in any case. The pathogens most resistant in the environment are helminth eggs, which in some cases can survive for several years in the soil. Pathogen survival in soil and on different crops can vary. For example, tapeworm eggs can survive in selected environmental media at 20-30 °C for many months in freshwater, sewage and soil and usually less than 30 days in crops,

which is the same for *Ascaris* eggs. Nevertheless, this pathogen can survive for years in freshwater, sewage and soil (WHO, 2006). Helminth eggs in wastewater were tested once during the study and 6 eggs 10 l⁻¹ were detected. Although the greatest health risks are associated with crops that are eaten raw, this value (6 eggs 10 L⁻¹) exceeds the WHO (1989) recommendation and Spanish legislation (RD 1620/2007) of ≤ 1 egg 10 l⁻¹. In this case, this water cannot be used to irrigate grass in a public park.

The threshold odor number (TON) is the greatest dilution of sample with odor-free water yielding a clearly perceptible odor (APHA, 1998). For the odor test, 1 ml of sample (wastewater) was diluted in 200 ml of odor-free water. All ten testers (100%) showed that wastewater odor was detectable. As 1:200 is the most diluted sample (APHA, 1998), it was not possible to prepare more diluted samples, and the TON resulted was 200 (Table 6). This means that treated wastewater from WWTP of Albacete is not advisable for public use as turf grass irrigation because of the odor nuisance.

Sample volumen diluted to 200 ml (ml)	TO N	Sample volumen diluted to 200 ml (ml)	TON
200	1	12.0	17
140	1.4	8.3	24
100	2	5.7	35
70	3	4.0	50
50	4	2.8	70
35	6	2.0	100
25	8	1.4	140
17	12	1.0	200

Table 6. Threshold odor numbers (TON) corresponding to various dilutions (APHA, 1998).

3.2 Soil

Table 7 shows that two months after stopping irrigation, no differences in pH were observed in soils between treatments or depths (0-25 cm, 25-45 cm). Organic matter increased at 0-25 cm in depth with respect to the original soil, while at the end of the study organic matter content at 25-45 cm in depth was lower in both types of soil. No important differences were observed in nitrogen content between treatments or depths, but a slight decrease at the end of the study was observed. C:N ratio increased for the two treatments in the 0-25 layer, but in the deeper layer it was the same as in the original soil. In general, nitric nitrogen in soils varies a lot, and in our case, at the end of the study was lower in all stratums than original soil. There were no differences in the 0-25 cm layer for both treatments at the end of the study, and at 25-45 cm in depth the value was lower than in the upper layer. At this depth, the nitric nitrogen level was higher in wastewater-irrigated soil than in the control soil. Phosphorus content in soil decreased at the end of the study respect to original soil. Two months after ceasing irrigation, wastewater-irrigated soil had higher phosphorus content than the control soil for the same depth and, to more depth, less phosphorus content in soil for the same treatment.

		November 2008			
PARAMETERS	UNITS	Control Plot	Wastewater Irrigated Plot	Control Plot	Wastewater Irrigated Plot
		0-25 cm		25-45 cm	
Sand	%	28	23	43	40
Silt	%	28	28	23	20
Clay	%	45	50	35	40
pH		8.57	8.91	8.45	8.51
E.C	mmhos cm ⁻¹	0.31	0.43	0.49	0.70
Chloride	(mg gypsum) (100 g soil) ⁻¹	44	59	68	133
Sulphate	(mg gypsum) (100 g soil) ⁻¹	37	115	163	188
Organic matter	%	3.6	3.7	1.8	2.0
Total N	%	0.15	0.15	0.17	0.13
C:N Ratio		14	14	7	9
Nitric N	mg kg ⁻¹	3	3	1	2
Asimilable P	mg kg ⁻¹	35	41	19	26
Assimilable K	meq 100g ⁻¹	2.05	2.47	1.02	0.98
Assimilable Na	meq 100g ⁻¹	0.59	2.04	0.64	2.20
Assimilable Ca	meq 100g ⁻¹	33.25	30.88	27.31	24.31
Assimilable Mg	meq 100g ⁻¹	6.09	6.40	4.46	3.95
Assimilable B	mg kg ⁻¹	2.40	5.60	2.39	2.40
K:Mg Ratio		0.34	0.39	0.23	0.25
Ca:Mg Ratio		5	5	6	6

Table 7. Chemical characteristics of the soil two months after ceasing irrigation at 0-25 cm and 25-45 cm in the control plot and wastewater-irrigated plot.

No changes in potassium soil content were observed throughout the study period but at the end of the study the deeper layer had less potassium. Magnesium increased in both types of treatment at 0-25 cm, and there were no differences between them for the same depth. In any case, magnesium content was higher at greater depth. A slight increase in calcium content at the end of the test was observed in the 0-25 cm layer with respect to the original soil. Hardly any differences between treatments were recorded, although calcium in the control soil was slightly higher.

No differences in K:Mg between treatments were observed for the same depth. This ratio remained stable in the 0-25 cm stratum but in at greater depth the value was lower. Finally, the Ca:Mg ratio remained constant throughout the study period but a slight increase at the end of the study at 25-45 cm in depth was observed. No differences between treatments were seen.

Usually, treated wastewater is more saline than tap water, and therefore, when it is reused in irrigation, more salinity problems can occur (Beltrao et al., 2003). Electrical conductivity decreased at the end of the study and was higher in the lower layer. In our case, the sodium content in irrigated water (170 mg l^{-1} in wastewater and 13.6 mg l^{-1} in control water) caused a noticeable increase of this salt in wastewater-irrigated soil at both depths studied. This must be kept in mind because sodium not only affects the soil structure, but may also have a toxic effect on plants. Previous research in this area showed E. C. stability (Mañas, 2006). In this study, an increase in sodium did not increase electrical conductivity, although several studies have shown that the higher the salt concentration is, the higher electrical conductivity is as well (Glober, 1996; Urbano, 2001; Mohammad and Mazahreh, 2003). On the other hand, sulphate content in the upper layer of the control soil decreased at the end of the study ($37 \text{ (mg gypsum) (100 g soil)}^{-1}$) but not in the rest of layers, so wastewater-irrigated soil had a higher sulphate level than control soil and in both cases sulphate concentrations were higher in deeper layers. Although there were no significant differences between sulphate content in wastewater and drinking water, higher sulphate was observed in the first 25 cm of soil irrigated with wastewater and also in the deeper layer for the two treatments at the end of the study. The sulphate ion causes no particular harmful effects on soils or plants; however, it contributes to increased salinity in the soil solution (Glober, 1996).

Otherwise, the most common phytotoxic ions that may be present in municipal sewage and treated effluents in concentrations such as to cause toxicity are boron, chloride and sodium (Pescod, 1992; Quian, 2008). The sodium content in the control soil was lower ($0.59 \text{ meq } 100\text{g}^{-1}$ at 0-25 cm and $0.64 \text{ meq } 100\text{g}^{-1}$ at 25-45 cm) than in wastewater-irrigated soil ($2.04 \text{ meq } 100\text{g}^{-1}$ at 0-25 cm and $2.20 \text{ meq } 100\text{g}^{-1}$ at 25-45 cm) in both layers analyzed. Chloride increased at the end of the study in both cases, and for the same treatment there was more chloride at greater depth. There is a scarcity of specific research on the function of boron in turf grasses. Plants differ in their boron requirements, with grasses generally having a much lower demand than dicotyledonous plants (Hull, 2002). In this study, boron content in the wastewater-irrigated plot was higher in the upper soil layer at the end of the study. Assimilable B content increased in the upper soil layer irrigated with wastewater (5.60 mg kg^{-1}). This fact could be the cause of phytotoxicity problems in several crops. In soils organic matter, Fe^{3+} and Al^{3+} oxides retain boron because of complex formation. This causes a very strong energetic fixation which makes it difficult for the plant to absorb boron. Maximum boron adsorption in soil occurs with $\text{Al}(\text{OH})_3$ at a pH of 8-9 (Urbano, 2001). At the end of our study, assimilable boron is more than double (5.60 mg kg^{-1}) in the wastewater-irrigated plot than in the control plot (2.40 mg kg^{-1}). Hull (2002) cites some experiences on the effects of managing turf using boron-contaminated irrigation water. In dry climates, boron from irrigation water can accumulate to concentrations of 10 ppm or greater in the soil. However, their research indicated that if turf is growing rapidly, it will dilute boron sufficiently that it will not reach toxic levels in plant tissues. In our case, the growth rate in the wastewater-irrigated plot was very fast. Therefore, since boron content in the irrigation water and soil after two years is not high we can deduce that the amount of boron in turf leaves did not increase to near toxic levels. As we explained previously, boron content in wastewater it is not a problem even for crops sensitive to boron. Hence, we do not consider boron an impediment for turf grass wastewater irrigation.

3.3 Turf grass

3.3.1 Height and phytomass

Grass height was recorded after the first mowing. Measurements show that the height of the wastewater-irrigated crop was always significantly higher than the control crop (Figure 6). The speed of growth after each mowing was higher for wastewater-irrigated grass as well.

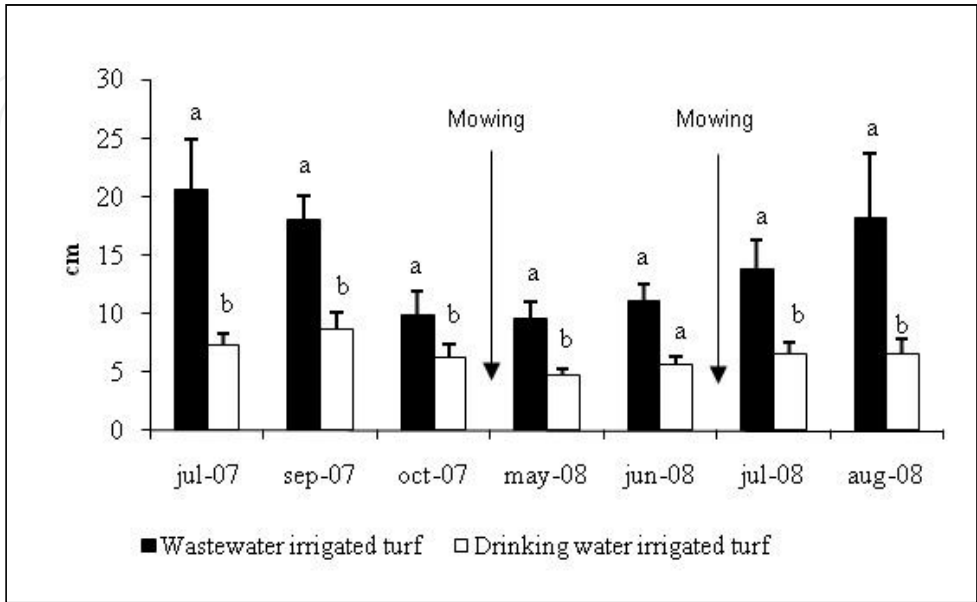


Fig. 6. Turf grass height (cm) throughout the study period.

During the winter season (October 2007-May 2008) the crop was not watered because grass growth ceased, resulting in lower height in both treatments. In July and August, 2008 (after the second mowing), the wastewater-irrigated grass continued growing until the end of the test, whereas the control grass maintained its height with no further growth reported.

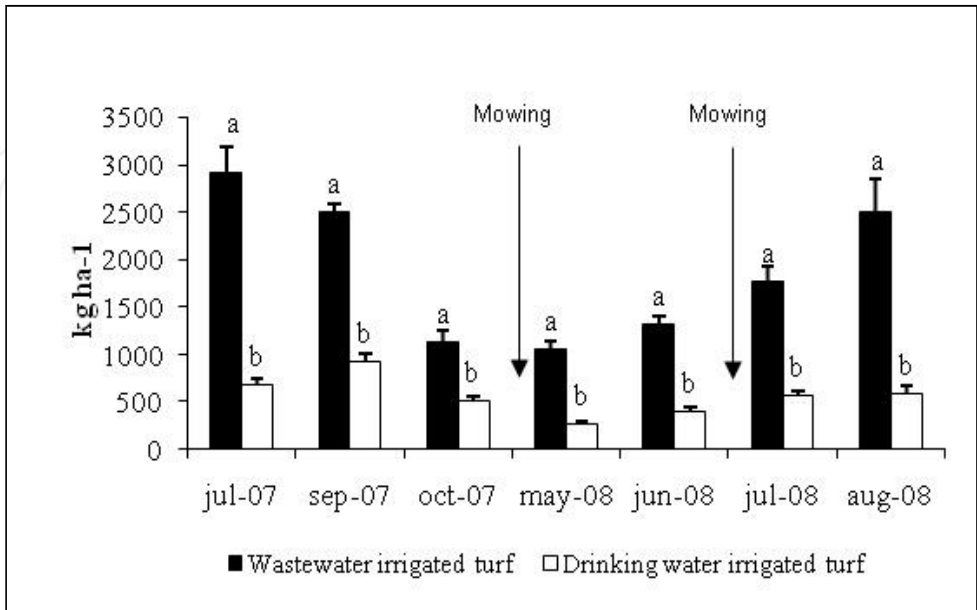


Fig. 7. Phytomass yield (kg ha⁻¹) throughout the study period.

The phytomass yield graph shows the same shape as the height graph (Figure 7). Growth in the wastewater-irrigated crop was always significantly higher than in the control crop. Studies have shown that continued use of irrigation water with electrical conductivity exceeding 0.75 dS m⁻¹ or total dissolved salts greater than 480 mg kg⁻¹ may reduce the growth and quality of turf grasses (Camberato, 2001). This is not the case in our study. Turf responded positively to irrigation with wastewater and reached greater height in addition to generating more biomass than turf irrigated with drinking water. Environmental conditions could substantially affect turf grass salt tolerance (Suplick-Ploense et al, 2002) and, in this case, the adequate nutrient content in irrigation wastewater, as shown below, and the selection of a resistant turf grass species avoided this problem. Regarding macronutrients in leaves, no significant differences in P, K and Mg foliar content were observed (Table 8) between wastewater-irrigated grass and the control.

Parameter	Units	Treated Wastewater	Drinking Water
		Average±sd	Average±sd
N	%	2.18±0.10 a	1.69±0.06 b
P	%	0.22±0.01 a	0.23±0.01 a
K	%	1.83±0.04 a	1.78±0.06 a
Ca	%	0.66±0.02 a	0.82±0.03 b
Mg	%	0.33±0.01 a	0.34±0.01 a
Na	mg kg ⁻¹	2291.50±66.78 a	280.47±9.08 b
Fe	mg kg ⁻¹	57.63±2.65 a	117.43±10.45 b
Mn	mg kg ⁻¹	52.65±1.85 a	56.15±0.26 a
Zn	mg kg ⁻¹	17.70±6.20 a	17.17±0.33 a
Cu	mg kg ⁻¹	4.93±0.12 a	4.70±0.08 a
Al	mg kg ⁻¹	53.53±3.89 a	146.63±17.67 b

Table 8. Chemical composition on Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, Sodium, Iron, Manganese, Zinc, Copper and Aluminium in turf grass (dw). Different letters mean significant differences between Treated Wastewater and Drinking Water at p<0.05, according to LSD test. (n=4).

Nevertheless, one of the most important differences with regard to the control turf grass was that turf grass irrigated with wastewater had greater N content (2.18% in wastewater irrigated turf grass and 1.69% in control plants) and less Ca in plant tissue (0.66% in wastewater irrigated turf grass and 0.82% in control turf grass). This higher nitrogen content in wastewater irrigated turf is directly linked with the higher phytomass yield obtained in this kind of turf. The present study was performed over two years, but other studies show that a longer period of wastewater application (10 years) resulted in lower biomass

production which nonetheless remained higher than that of the control plants (Mohammad et al., 2007). Thus, periodic monitoring of soil and plant quality parameters would be recommended to ensure successful, safe, long-term wastewater irrigation.

With respect to micronutrients, it is important to emphasize the behavior of Na because foliar tissue from the wastewater-irrigated plot is much higher (2291.5 mg kg⁻¹) than the control (280.5 mg kg⁻¹). Thus, Na in wastewater-irrigated turf grass leaves was 8 times higher with respect to the control (717% higher). This may be an effect of sprinkler irrigation, as sodium and chloride frequently accumulate by direct adsorption through leaves that are moistened (Quian, 2008).

Fe and Al foliar content was significant lower in wastewater-irrigated grass than in the control. This could be because Fe and Al are fixed by organic matter in the soil, and the plant cannot take them up. Finally, no significant differences were observed for Mn, Zn and Cu.

If the plants were grown for raw consumption, heavy metal contamination of urban agricultural fields under long-term application of wastewater could be problematic, and there are many studies on this topic (Mañas, 2006; Castro, 2007; Agbenin et al., 2009). However, this is not the case in the present study.

Regarding microbiological parameters, the main factors that affect pathogen survival in the environment are humidity, soil content, temperature, pH, sunlight (ultraviolet radiation), foliage and competition with native flora and fauna; pathogen inactivation is much more rapid in hot, sunny weather than in cool, cloudy and rainy conditions and low temperatures prolong pathogen survival (WHO, 2006).

Sunlight and temperature are parameters with a high influence on the dynamics of microbes, and in our study we can see that the slopes of the mean average temperature and sunlight (Figure 3) fit into a multiplicative model (Equation 2). Y= mean average temperature; X= Sunlight:

$$\ln(Y) = -1.71244 + 1.46425 * \ln(X) \quad (2)$$

$$R^2 = 90.52\% \text{ and } P\text{-value} < 0.001$$

Since sunlight has a strong correlation with temperature, we can select either of the two variables to study the evolution of pathogens. Hence, Figures 8, 9, 10 and 11 show the dynamics of microorganisms related to sunlight in a regression analysis in which independent variable is “days after planting” and cfu count is considered dependent variable. It is known that high temperatures lead to rapid mortality and low temperatures lead to prolonged survival, while freezing temperatures can also cause pathogen mortality. Direct sunlight leads to rapid pathogen inactivation through desiccation and exposure to ultraviolet radiation (WHO, 2006).

In the present study, sunlight decreased from July 2007 to January 2008 and increased from then until the end of August, 2008 (Figure 3). *Salmonella sp* was not detected in leaves at any time. Total coliforms and faecal streptococci in turf grass leaves (Figures 8 and 10) increased with both types of irrigation (wastewater and control). Faecal coliforms (Figure 9) also increased but the trend was more stable than the trend for the microbes mentioned above.

The unchanging increase in microorganisms could be explained because in spring-summer time, constant irrigation of turf with both types of water led to microbial growth despite the

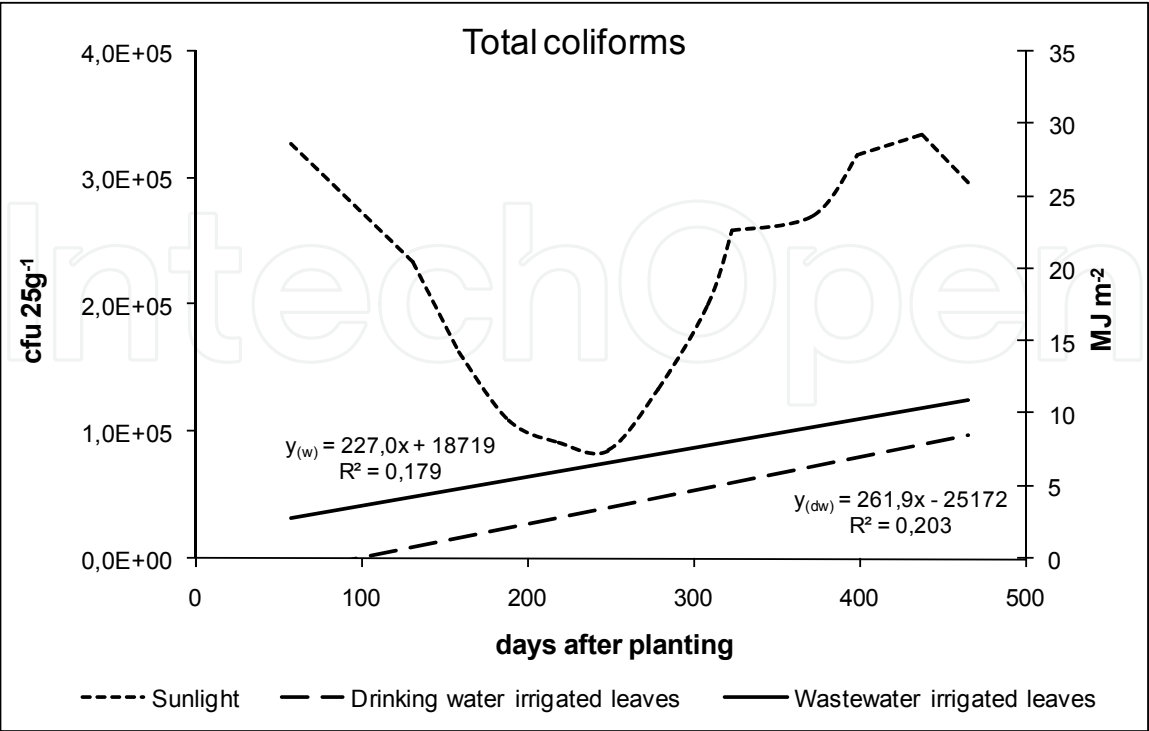


Fig. 8. Dynamics of total coliforms in turf grass leaves irrigated with drinking water versus wastewater and sunlight during the study period.

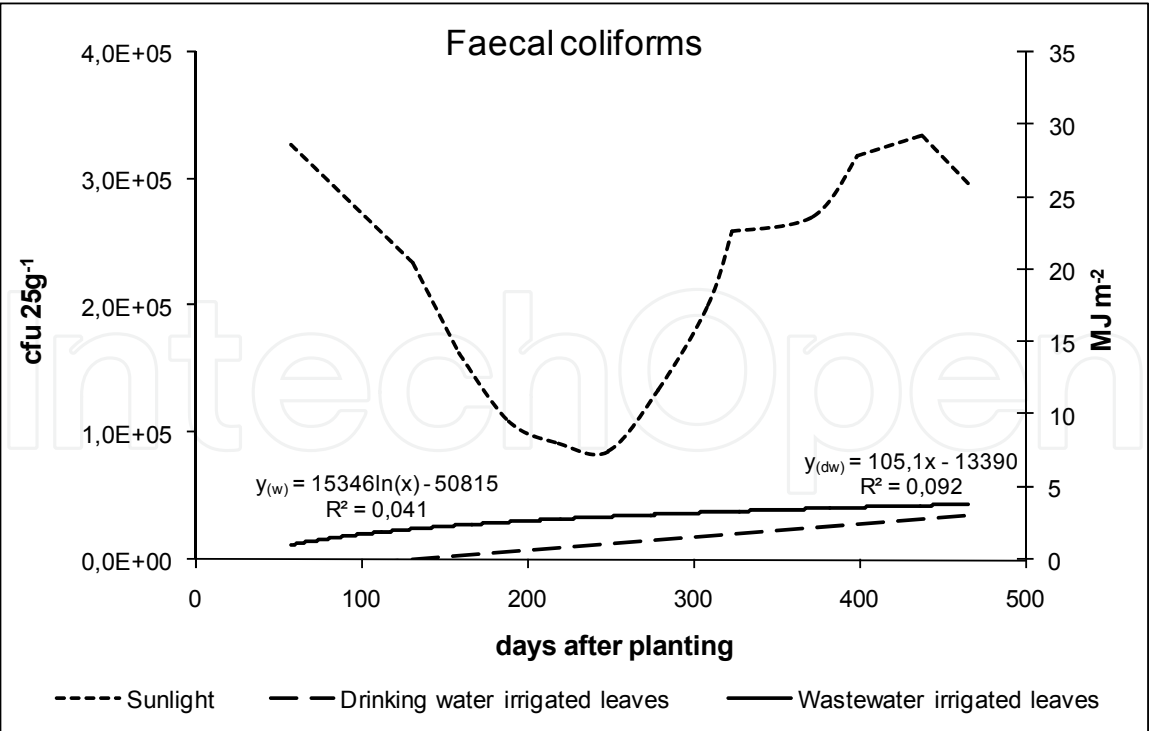


Fig. 9. Dynamics of faecal coliforms in turf grass leaves irrigated with drinking water versus wastewater and sunlight during study period.

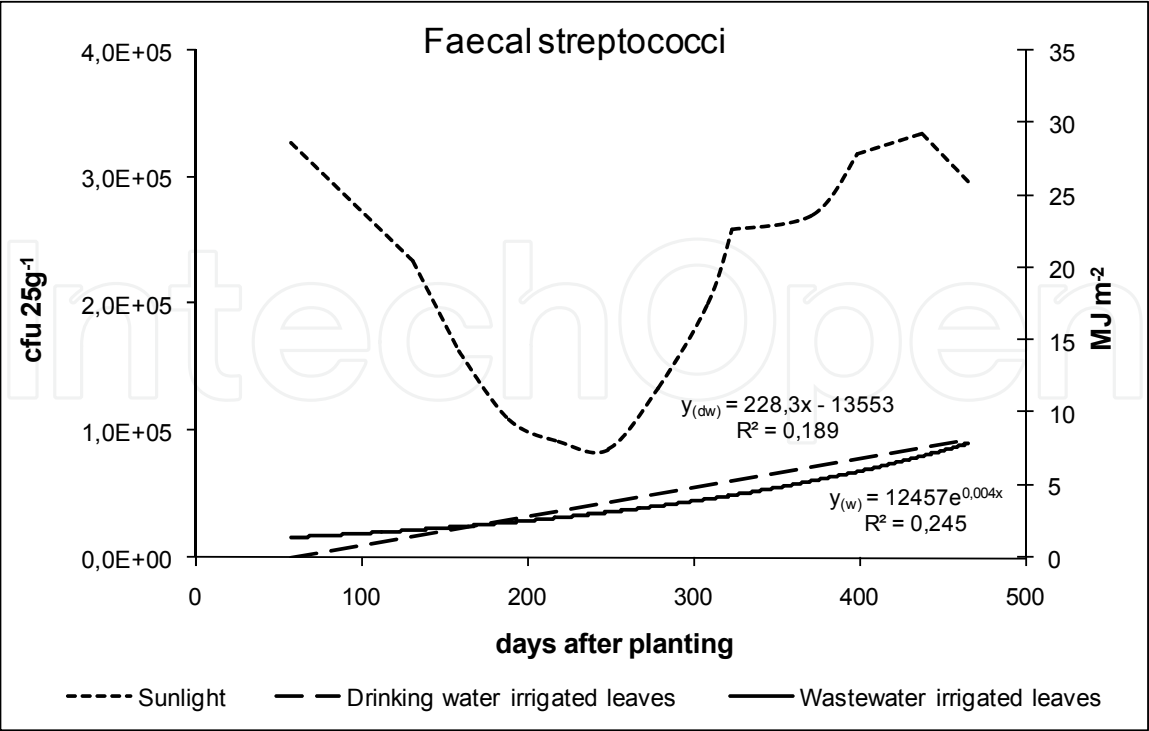


Fig. 10. Dynamics of faecal streptococci in turf grass leaves irrigated with drinking water versus wastewater and sunlight during study period

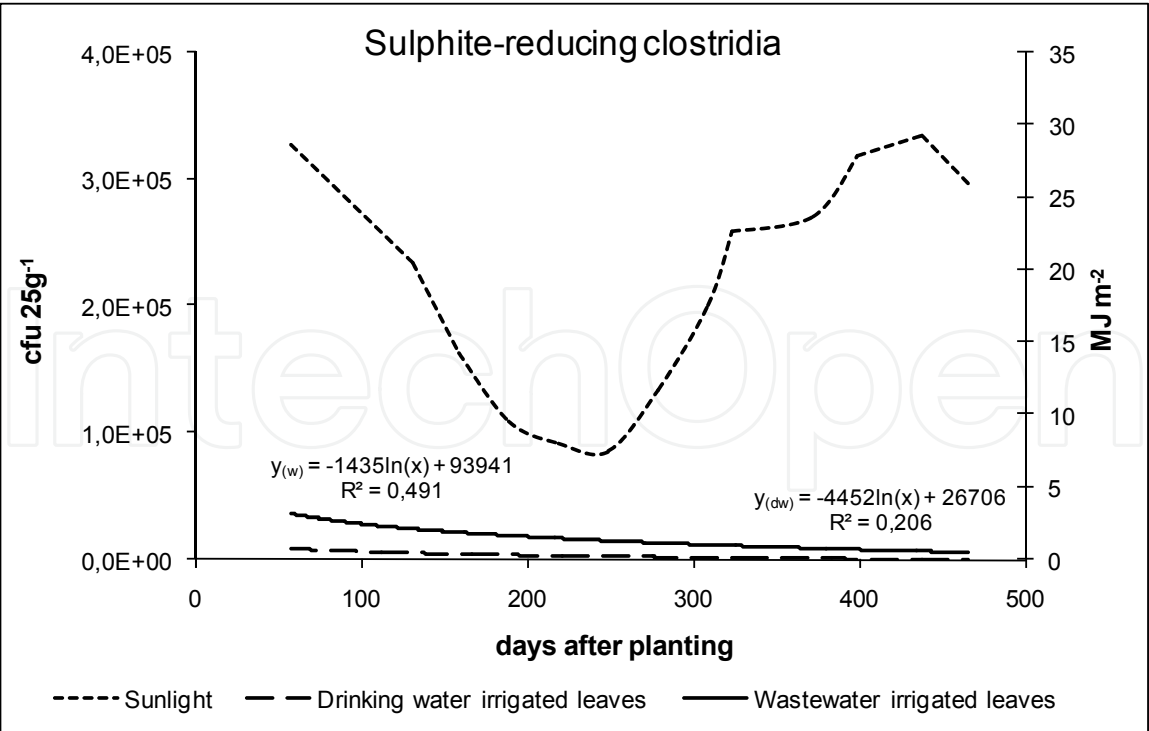


Fig. 11. Dynamics of sulphite-reducing Clostridia in turf grass leaves irrigated with drinking water versus wastewater and sunlight during study period.

negative effects of sunlight (ultraviolet radiation) and high temperature on pathogens. In the autumn-winter seasons, sunlight decreased but low (but not below zero) temperatures and humid environmental conditions caused by rainfall that year (not from irrigation in this case) favored pathogen survival.

By contrast, sulphite-reducing Clostridia decreased throughout the study (Figure 11). In our case, constant irrigation maintains microbiological population on the leaves. Although the dynamics of microorganisms showed parallel curves, the slope of microbes from wastewater-irrigated turf grass was higher than the control in the case of total and faecal coliforms and sulphite-reducing Clostridia (Figures 8, 9 and 11). In contrast, Figure 10 showed no significant difference in faecal streptococci content in the two treatments.

We can deduce that the dynamics of total and faecal coliforms and sulphite-reducing Clostridia were the same for both types of irrigation water but the continuous entry of microorganisms in plots with wastewater irrigation allowed for a larger population.

4. Conclusions

To conclude our study we have found some advantages and some disadvantages in using wastewater for irrigation turf grass. It is clearly noticeable that some of those advantages are:

- Physical and chemical parameters evaluated in our study showed that the use of treated wastewater for irrigate turf grass was useful to get improve in its growth, especially in terms of height and phytomass.
- No negative effects with respect to changes in soil pH, electrical conductivity or salinity occurred.
- Treated wastewater from the Albacete wastewater treatment plant can be a source of fertilizer since it has an important contribution of N, P and organic matter and can save farmers money on fertilizer.
- In foliar tissue, *Salmonella sp.* was not detected in any case and sulphite-reducing clostridia content did not increase throughout the study period.

However, it is necessary to be aware that several crops could suffer from the negative effects of wastewater irrigation due to the sodium and boron content and should keep this in mind that the benefits of irrigation with treated urban wastewater including addition of plant nutrients to turf grass and conservation of valuable freshwater resources.

In contrast, some risks for human health arise from microbiological aspects also evaluated in this study:

- Helminth egg content in wastewater irrigation results were 6 eggs 10 l^{-1} and exceeded the limit recommended by the WHO and Spanish legislation.
- One hundred percent of testers agreed that wastewater odor was perceptible at a 200 ml dilution.
- Coliforms (total and faecal) and faecal streptococci have a tendency to increase throughout the study.

In spite of the commented advantages and according to the odor test and microbiological parameters results it is not advisable to use wastewater irrigation from the wastewater treatment plant of Albacete since it could be harmful in a public garden. It is essential to disinfect this wastewater and remove the odor with appropriate advanced treatments at the end of the process before using it.

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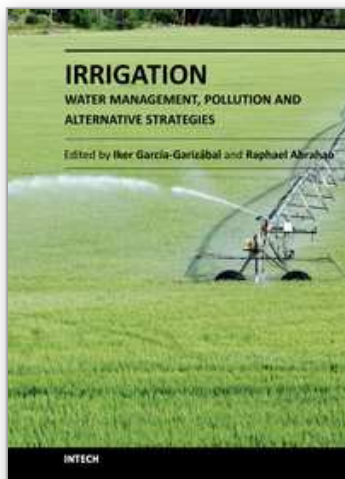
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Irrigation - Water Management, Pollution and Alternative Strategies

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Irrigated agriculture is the most significant user of fresh water in the world and, due to the large area occupied, is one of the major pollution sources for the water resources. This book comprises 12 chapters that cover different issues and problematics of irrigated agriculture: from water use in different irrigated systems to pollution generated by irrigated agriculture. Moreover, the book also includes chapters that deal with new possibilities of improving irrigation techniques through the reuse of drainage water and wastewater, helping to reduce freshwater extractions. A wide range of issues is herein presented, related to the evaluation of irrigated agriculture impacts and management practices to reduce these impacts on the environment.

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